silica were geometrically shielding the carbon, fluidized bed combustion could have yielded in better carbon conversion since it improves the comminuting of particles in the bed, thus exposing fresh surfaces for gasification. It appears that silica forms molecular bonds with carbon, which are not easily broken at the gasification temperatures. Reactions leading to the formation of silicon carbide from silica involve very high temperatures (above 2500°C) and gasification temperatures are not high enough for such reactions to take place.

In view of these results, we suggest that full carbon conversion in rice husk may not be achievable. However, this does not diminish the importance of rice husk as a fuel source because of its widespread and large availability at affordable prices.

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Accumulation of the periplasmic protein alkaline phosphatase in cell cytosol induces heat shock response in *E. coli*

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When the cells of *Escherichia coli* mutant MPh1061 (the mutation is at the N-terminal signal sequence part of the alkaline phosphatase [AP] gene) were grown in phosphate-less medium, accumulation of the AP precursor (AP with its signal sequence) in the cell cytosol led to induction of cellular heat shock response. The autoradiograph of the SDS-polyacrylamide gel electrophoretic pattern of the proteins extracted from the ^{35}S-methionine-labelled cells showed enhanced synthesis of different heat shock proteins. This was further confirmed by the immuno-precipitation study using the antibodies of the heat-shock chaperones, GroEL and GroES. Our experimental results suggest that the cytoplasmic accumulation of inactive AP behaved as abnormal protein to the cells, which triggered the induction of heat shock response.

IN *Escherichia coli*, all periplasmic and outer membrane proteins are synthesized in the cytosol initially in precursor form with an amino-terminal signal sequence of 18–26 amino acids[1]. The signal sequence binds to the translocation machinery on the inner membrane and guides the precursor protein into the membrane for translocation[2]. Moreover, interaction with cellular chaperones maintains these precursor proteins in a translocation-competent unfolded conformation until they are transported through the inner cytoplasmic membrane by the translocation machinery[3]. After the precursor proteins enter the periplasm, the signal sequence is ultimately removed by signal peptidase, an integral membrane protein with the active site facing the periplasm[4]. From analyses of the mutants, it is observed that a functional signal sequence is required for export; for proteins whose signal sequences have been mutationally altered, their precursors accumulate in the cytoplasm instead of being exported out across the inner membrane[5-7]. In this study we have examined the expression of alkaline phosphatase (AP) in *E. coli* MPh1061 strain carrying a mutationally altered signal sequence (Leu-14 → Arg) to determine how *E. coli* cells respond to the storage of inactive AP precursor in cell cytoplasm by inducing cellular stress response.

The bacterial strain *E. coli* MPh42 and its AP signal sequence mutant[5] MPh1061, used in this study, were

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obtained from Dr Jonathan Beckwith, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, USA.

All the components of the growth medium and the electrophoresis reagents were purchased from Amersham Pharmacia Biotech. For immuno-precipitation study, antibodies to GroEL and GroES were procured from Sigma and Protein A-CL agarose from Bangalore Genei Pvt Ltd. ^{35}S-methionine, used to study protein synthesis, was bought from Board of Radiation and Isotope Technology, India.

To study the export deficiency of AP, cells were initially grown to log phase [up to $(OD)_{600\ nm} \approx 0.3$, i.e. $\sim 1.5 \times 10^8$ cells/ml] at 30°C in MOPS medium[8], where the methionine concentration was one-tenth of that in normal MOPS medium. The grown cells were centrifuged, washed with and finally suspended in phosphate-free and mithonine-free MOPS medium. In the suspended cell culture, 35S-methionine was added at a concentration of 30 µCi/ml – the cells were then allowed to grow and were labelled at 30°C for a pulse of 90 s, the labelling was subsequently chased by 0.2 M cold methionine. At 0 and 10 min of chasing, cell aliquots of 0.5 ml were withdrawn to extract proteins, according to the method of Oliver and Beckwith[9] for immunoprecipitation. To the extract, 3.0 µl of antiserum to AP was added and the mixture was kept on ice to incubate overnight at 0°C. To this incubated sample, 60 µl of protein A-CL agarose was added and further kept on ice for 20 min. The immunocomplex was washed and finally suspended in 50 µl DTT-free gel loading buffer[9] to keep and run the protein in 10% SDS-polyacrylamide gel[10] under non-reducing condition. For autoradiography, the gel after electrophoresis was fixed in 12% glacial acetic acid and 30% methanol solution for 30 min, rinsed in distilled water and soaked in 1M sodium salicylate (E. Merck) for 30 min[11]. Gels were then dried and exposed on X-ray film (Kodak X-Omat) at – 70°C for 48–72 h. In this study, the AP antibody was raised according to the method of Oliver and Beckwith[9] by injecting a rabbit interdermally at four sites with AP (Sigma; 100 µg per rabbit) emulsified with complete and incomplete Freunds adjuvant (Sigma) for the primary injection. Two subsequent injections of 50 µg AP and one booster dose of 100 µg AP, each emulsified with incomplete Freunds adjuvant, were pushed at intervals of 10 days. After three days of injecting the booster dose, blood was drawn from the marginal vein of the ear.

For the study of heat shock proteins (HSPs), log phase-grown cells in MOPS medium, as described above, were divided in three equal parts and harvested. One part was resuspended in phosphate-free MOPS medium and was allowed to grow further at 30°C. The other two parts were separately resuspended in normal phosphate-containing medium; one part was grown at 30°C, while the other was allowed to grow at 50°C. After 30 min of growth, ^{35}S-menthionine (15 µCi) was added to 0.5 ml each of the three growing cultures to label the cells for 10 min. Cells were then chilled, harvested and proteins were extracted by boiling the cells with SDBME buffer[8]. Equal amounts of protein from the cultures, estimated by the Bradford method, were loaded on 12% SDS-polyacrylamide gel for electrophoresis. The autoradiography of the gel was done according to the method described above and the exposed X-ray film was scanned by the gel documentation software, Quantity One (BIORAD).

To identify the HSPs GroEL and GroES by the immunoprecipitation technique, cells were grown and labelled with ^{35}S-methionine as described in the study of HSPs and then the proteins were extracted from the cells, according to the method of Oliver and Beckwith[9]. The immunoprecipitation study of the protein extract was performed as described above using 1.0 µl of antibody to GroEL and 3.0 µl of antibody to GroES. The immuno-complex was washed and finally suspended in 50 µl gel-loading buffer[9], prior to electrophoresis in 12% SDS–polyacrylamide gel.

In this study our motivation was to observe how *E. coli* responds to the accumulation of a membrane protein in the cell cytoplasm. For this purpose, the periplasmic protein AP of *E. coli* was selected. AP, a nonspecific phosphomonoesterase, can generate inorganic phosphate from a variety of phosphorylated derivatives. Its synthesis is regulated by end-product repression, i.e. the addition of phosphate to the media inhibits expression of the AP gene; in other words, phosphate starvation induces AP synthesis[12]. Therefore, growth of *E. coli* cells in phosphate-free medium induces the expression of AP gene. AP is synthesized initially as a precursor molecule having a signal sequence of 21 amino acids at its amino terminus, which guides the nascent polypeptide to the cytoplasmic membrane and the signal sequence is ultimately removed proteolytically by signal peptidase, after the protein is exported out through the membrane[7]. After cleavage of the signal sequence, the enzymatically active AP is localized in the periplasm as a dimer; dimerization occurs through formation of intra-chain disulphide bonds[13]. AP cannot be enzymatically active when it is retained in the cell cytoplasm specifically because its disulphide bonds cannot ordinarily be formed in the reducing environment of the cell cytosol[14,15].

The *E. coli* mutant used in our study was such that there was a point mutation at the signal sequence region of the AP gene. Due to the mutation, when the cells were allowed to grow in phosphate-less medium, the induced AP could not be exported out to the periplasm across the inner cytoplasmic membrane; instead the AP precursor accumulated in the cytoplasm. We checked this export deficiency of AP precursor in MPh1061 mutant cells, as was done by Michaelis *et al.*[5], by the pulse-chase experiment with ^{35}S-methionine and the subsequent immunoprecipitation study with AP antibody, such that the precursor and the secreted mature species of AP were

resolved. The maturation of AP, i.e. the cleavage of signal peptide as well as the intra-chain disulphide bond formation was reported[7,13] to occur only after a protein had translocated through the membrane. Therefore, by the run in SDS–polycrylamide gel under non-reducing condition, shown in Figure 1, the mature form (signal sequence-less and folded by intra-chain disulphide bond) moved faster than the precursor form (signal sequence-containing and having no intra-chain disulphide bond). Figure 1 signifies that under the condition of induction of AP, when *E. coli* cells were allowed to label with ^{35}S-methionine for a pulse of 90 s and then the labelling was subsequently chased with cold methionine for 10 min, the nascent AP precursor of the mutant MPh1061 cells was not matured during the chase period, while the maturation occurred in the wild type MPh42 cells. Figure 1 also shows that for wild-type AP, after a 90 s pulse label (with no chase period), the radiolabelled product was in the mature form; no precursor could be detected. This rapid and efficient removal of the signal peptide from nascent AP molecules of wild-type cells indicated that the translocation of AP across the cytoplasmic membrane occurred co-translationally. However, the above result implied that in the signal sequence mutant strain *E. coli* MPh1061, the AP was induced by its growth in the phosphate-less medium; but the induced AP precursor could not be exported out from the cell cytosol. Moreover, when the enzymatic activity of AP of the mutant cells was assayed, it showed zero activity (data not shown) due to lack of maturation of AP.

In this communication we report that as a consequence of the cytoplasmic storage of AP precursor, HSPs were induced as cellular response. When cells of *E. coli* strain MPh1061 were allowed to grow and label with ^{35}S-methionine at 30°C in phosphate-free MOPS medium, syntheses of some proteins, as observed from the autoradiographic pattern of the bands of proteins of labelled cell extracts electrophoresed in SDS–polyacrylamide gel (Figure 2), were enhanced (bands marked by dots in lane *d*, Figure 2) with respect to those in control cells grown at the same 30°C temperature in phosphate-containing MOPS medium (lane *c*, Figure 2). Compared with proteins of cells grown and labelled at 50°C (where most

cellular proteins, except HSPs, ceased to be synthesized[16]) in phosphate-containing medium (lane *b*, Figure 2), the highly synthesized protein bands in lane *d*, Figure 2 were found to be of HSPs. Moreover, comparing the mobilities of the HSPs with those of the molecular weight marker proteins, the HSPs in lane *d* seemed to be as Lon, DnaK, GroEL, DnaJ, small HSPs, IbpAB and GroES serially from the top to the bottom of the gel. Such induction of HSPs was not observed in *E. coli* MPh42 cells (where there was no mutation at the AP signal sequence) by their growth in phosphate-less medium (lane *a*, Figure 2). Therefore, the above result shows that the growth of the AP signal sequence mutant *E. coli* MPh1061 in phosphate-starved medium (where AP was induced), led to the induction of heat shock chaperones DnaK, DnaJ, GroEL, GroES, heat shock protease Lon and small HSPs IbpAB (lane *d*, Figure 2) over their normal level present in the same cells (lane *c*, Figure 2), when grown in phosphate-containing medium (where AP expression remained repressed). Normal levels of HSPs, represented by the faint bands (except GroES) in lane *c*, Figure 2, are known to operate under all growth conditions to cope with *de novo* protein folding and quality control, and becomes particularly important under stress conditions such as
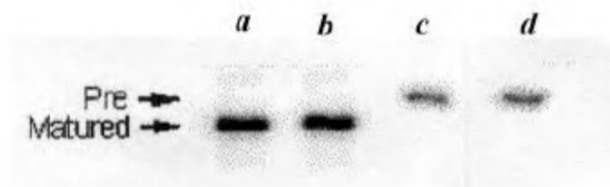

Figure 2. Autoradiograph of 12% SDS–polyacrylamide gel (regular in size) electrophoretic pattern of the protein extract from ^{35}S-methionine-labelled cells. Lane *a*, MPh42 cells grown at 30°C in phosphate-less medium; lane *b*, MPh1061 cells grown at 50°C in phosphate-containing medium; lane *c*, MPh1061 cells grown at 30°C in phosphate-containing medium and lane *d*, MPh1061 cells grown at 30°C in phosphate-free medium.

Figure 1. Autoradiograph of pulse-chase analysis to study AP export in wild type and signal sequence mutant cells. The 10% SDS–polycrylamide gel was of regular in size (16 cm × 14 cm). Lane a, MPh42, chased for 0 min; lane b, MPh42, chased for 10 min; lane c, MPh1061, chased for 0 min and lane d, MPh1061, chased for 10 min.

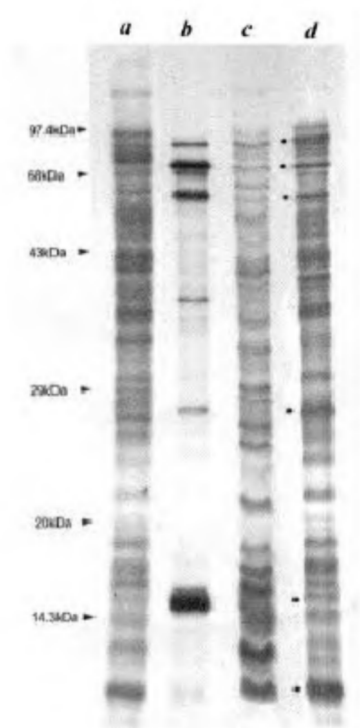heat shock during which they are also induced[17]. By the growth of the cells in the plus-phosphate medium at 50°C, the HSP bands were found to be more intense (lane *b*, Figure 2) than those in lane *c*, and the bands in lane *b* indicated near exclusive synthesis of HSPs at such lethal temperature. Comparison of the bands between lanes *b* and *d* clearly showed that the growth of AP export-deficient cells in phosphate-less medium (i.e. under the condition of induction of AP) caused enhanced synthesis of HSPs. This induction of HSPs might be interpreted as a consequence of cellular stress caused generally by the overproduction of AP, instead of cytosolic storage of AP precursor due to its export deficiency. However, this was found to be wrong, as the growth of the wild-type MPh42 cells (where AP was induced as well as exported) in phosphate-free medium showed no enhanced synthesis of HSPs (lane *a*, Figure 2).

To confirm the induction of heat shock response (at least at the levels of GroEL and GroES) in a more deterministic way, immunoprecipitation study was performed using the antibodies of GroEL and GroES. The result in Figure 3 was positive, i.e. the amount of precipitate of GroEL and GroES by their antibodies from the extract of MPh1061 cells grown in no-phosphate medium (lane b, Figure 3) was considerably higher than those from cells grown in phosphate-medium (lane a, Figure 3), which implied the presence of much more GroEL and GroES in the former cells than in the latter ones. It appears from Figure 2 that the amount of cellular GroES did not differ much with change in the growth medium – with or without phosphate. On the contrary, quite the opposite result came out from the more confirmatory immunoprecipitation study (Figure 3), i.e. much more GroES was synthesized in cells when there was no phosphate in the growth medium. This difference in results with respect to GroES induction can be interpreted as the superposition of other 10 kDa cellular proteins with GroES, which might be responsible for the formation of intense band at the position of GroES in lane *c*, Figure 2.

The induction of HSPs in *E. coli* due to storage of inactive AP precursor in cell cytosol can be explained from the fact that the synthesis of abnormal proteins in *E. coli* at normal growth temperature led to slow but permanent generation of heat shock response[18,19]. Furthermore, the presence of partially denatured and aggregated proteins in cells also elicited induction of HSPs, which by their chaperonic and/or protease activities collectively function to protect the cells from stressful conditions and to maintain the conformational stability of cellular proteins[20,21]. Our results are in conformity with the observations of Betton *et al.*[22], who showed that the cytoplasmic accumulation of maltose-binding protein (the periplasmic receptor for the high affinity transport of maltose) had increased the synthesis of DnaK and GroEL. Moreover, it was reported[23] that the moderate deficiency in signal recognition particle (a ribonucleoprotein complex that
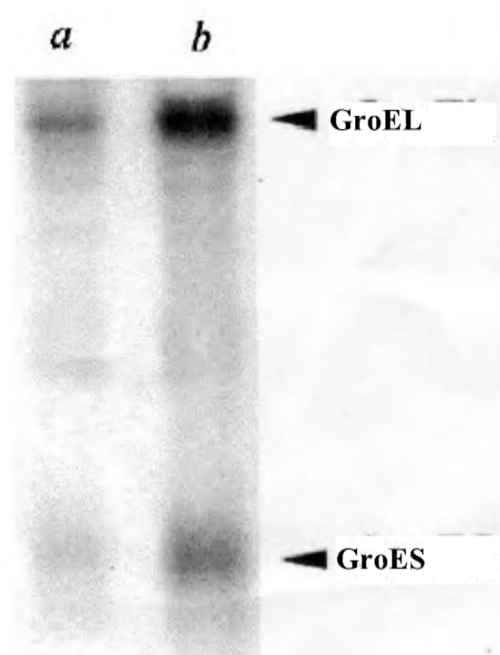

Figure 3. Autoradiograph of 12% SDS–polyacrylamide gel (mini: 8.3 cm × 7.3 cm) electorphoretic pattern of immunoprecipitates using GroEL and GroES antibodies from protein extract of ^{35}S-methionine-labelled cells. Lane a, Cells grown at 30°C in phosphate-containing medium; lane b, Cells grown at 30°C in phosphate-free medium.

targets inner membrane proteins to their proper membrane locations) induced heat shock response and the heat shock-regulated protease. Lon became essential to maintain cell viability by degrading the inner membrane proteins mislocalized in the cytoplasm. Even the formation of inclusion bodies in recombinant *E. coli* elevated the synthesis of HSPs[24]. In the light of the above findings, we propose that the accumulated inactive AP in the cell cytoplasm behaved as abnormal proteins to which *E. coli* cells responded by inducing heat shock response.

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On the recurrence of *Noctiluca scintillans* bloom in Minnie Bay, Port Blair: Impact on water quality and bioactivity of extracts

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A *Noctiluca scintillans* bloom in the coastal waters of Minnie Bay, Port Blair was studied. Physico-chemical and biological properties of bloom-infested waters were monitored during the bloom period lasting five days. The bloom appeared as a green streak along the entire coastline of Minnie Bay, with cell counts of 17×10^3 cells/l. The bloom appeared as a sudden spurt in cell number and persisted for a period of 48 h. The antibacterial properties of extracts from this algal species were also investigated. Conspicuously, the bloom inhibited the common resident phytoplankton species. Total suspended solids showed a marked increase during day-one of bloom compared to ambient levels. The bloom appeared to be limited by dissolved inorganic nitrogen species availability. The differential growth of phytoplankton reveals the involvement of specific trigger factors for such blooms. From the present viewpoint, micro-scale studies on hydrobiological factors preceding the onset of bloom would reveal what cycle of events lead to a bloom and the causal factors of such blooms. However, prediction of occurrence of such blooms and *in situ* measurements are practical difficulties to be addressed. Since a similar bloom was reported earlier in 2001, it is worthwhile to keep a watch and investigate as to whether there is any anthropogenic or environmental cause for the recurrence of the bloom.

NOCTILUCA represents a substantial fraction of bloom-forming marine dinoflagellate population of the world's oceans. This bioluminescent dinoflagellate is known to light the wakes of boats and the breaking waves on beaches. Occurrences of *Noctiluca* bloom in coastal waters of the Indian subcontinent are not uncommon phenomena. However, the causes for the occurrence of *Noctiluca* bloom in Indian coastal waters are not well understood. The increased frequency of appearance of blooms throughout the world in the recent past has led some scientists to believe that a change in the marine planktonic ecosystems on a global scale is caused by human alterations to the coastal zone. Algologists have long grappled with the fundamental question of what factors determine the differential growth

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